Hieracium on the other. The doubling of chromosomes, the terminology of parthenogenesis, the nucellar embryos, the lessened fertility and many other effects of hybridizing, as well as those of vegetative propagation are extensively dealt with. From this survey the author concludes that *Chara crinita* seems to afford the best material for further studies and gives an ample review of the mode of propagation of this algæ.

It is a dioecious plant, which has a parthenogenetic variety. The latter has been described by *Alexander Braun* as early as 1856 and since by numerous authors. The species is rather rare; in some stations it is found without the variety but in the larger number of localities only the apogamous form occurs. In some, however, both grow together, indicating the possibility of a repeated origin of the variety from the dioecious type. Moreover it is shown that the differences between the two types are of such a kind, that they can not have originated slowly and gradually but must be assumed to be due to a sudden change (p. 104). This is the well-known way in which in other cases mutations are seen to arise. The probable difficulties of the intended investigation are then amply discussed. To these the reviewer might add the objection that it is a species which has already produced an apogamous form, and probably more than once and which therefore may be expected to repeat the mutation from time to time, even without the aid of experimental interference. Furthermore, the experience with the evening primrose has shown that mutations occur in crossed progeny as well as in pure lines and the research of Baur on *Antirhinum* and of Morgan on *Drosophila* have amply confirmed this result. Among hybrid progenies they seem to be more numerous, but only in consequence of the fact that such cultures usually embrace many thousands of individuals more than are kept in the pure stocks. The same will be the case in the cultures of *chara crinita* and the expected occurrence of apogamous mutations in hybrid families can, therefore, not be regarded as a proof of their origin by means of hybridization.

But it seems highly desirable that the experimental trials should be made, the more while in any case the gain for the theory of mutation must be expected to be of the highest importance.

Hugo de Vries

Lunteren, Holland

PRELIMINARY REPORT OF EXPERIMENTS ON THE ACTION OF DICHLOROETHYLSULFIDE (MUSTARD GAS) ON THE CELLS OF MARINE ORGANISMS[1]

The toxic action of a sample of "mustard gas" sent us by Major H. C. Bradley, of the Chemical Warfare Service, has been investigated on a number of typical marine organisms, including various swimming larvæ (sea-urchin, starfish, squid, the annelids *Nereis* and *Arenicola*), the developing eggs of sea-urchin and star-fish, the spermatozoa of sea-urchin and starfish, and young and adult fish (*Fundulus*). The most satisfactory objects for experimentation have proved to be the developing eggs of the starfish (*Asterias forbesii*), and most of our work has been carried out with this material. Changes in the rate and character of cleavage in the eggs after treatment with "mustard," the production of abnormalities of form and structure in the larvæ, and the degree of ciliary activity, furnish a very delicate index of toxic action. Valuable information has also been obtained with *Arenicola* larvæ and with small fish (*Fundulus*).

In the experiments with fertilized starfish eggs we have investigated the influence of solutions of the "mustard gas" in sea-water upon the cleavage and early development (up to the gastrula stage). The procedure chiefly employed was as follows: A small quantity of the "mustard gas" (ca. 5 grams) was shaken vigorously with one liter of sea-water in a

[1] This preliminary report in its present form was sent to the Medical Section, Chemical Warfare Service, September, 1918. A more detailed account of these experiments will be published in the near future.

2-liter glass-stoppered bottle. After the finely divided undissolved oil had settled, the clear liquid from the middle of the solution was drawn off, and the action of this saturated solution upon the recently fertilized mature eggs was tested, using varying dilutions (*e. g.*, 1/2, 1/4, 1/8, 1/16 saturated) and varying times of exposure (from one fourth minute to an hour or more). The eggs were exposed to the solutions in glass-stoppered bottles, and at intervals portions were transferred by pipette to dishes of normal sea-water; this water was changed when the eggs had settled. The subsequent course of cleavage and development, as compared with that of untreated "control" eggs, was carefully studied.

The toxicity of "mustard" solutions prepared in the above manner is not constant but decreases with standing, and the more rapidly the higher the temperature. Solutions made at room temperature (20–24°) always prove strongly toxic if used immediately after preparation; if used later the toxic action is less marked, the decline of toxicity being rapid in the first hour and more gradual later. This decline is due to the progressive hydrolysis of the "mustard," which breaks down rapidly in aqueous solution, yielding HCl and residual compounds of low toxicity. The toxicity of a "mustard" solution two days old, in which the acid freed is neutralized by NaOH, is not more than one fiftieth of that of the freshly prepared solution, as measured by the comparative times of exposure required to produce a definite impairment of development or a definite proportion of dead eggs in a given time. The attenuation of toxicity, as thus shown by the physiological action of the solution, exhibits a general parallelism with the production of HCl, as measured by titration (with dibromocresolsulphonephthalein as indicator). The essential toxic action is thus due to the undecomposed "mustard" in the solution. This conclusion was confirmed by experiments in which the hydrolysis of the compound was retarded by cold. The oil was shaken with ice cold sea-water (below 3°), the solution was filtered free from the residual undissolved crystals of "mustard" (which is solid at this temperature), and the cold saturated solution thus obtained was kept at 0° (surrounded by ice in the refrigerator). The toxic action of a portion of the solution kept thus cold and brought to room temperature immediately before adding the eggs was compared with that of portions which were brought to room temperature and allowed to stand for varying times (*e. g.*, 1/4 hour, 1/2 hour, 3 hours, 24 hours) before using. In all cases solutions which were kept cold until just before using were decidedly the most toxic, 15 minutes' exposure to room temperature reduces toxicity by about one half, and 30 minutes by two thirds or three quarters. The decline in toxicity is thus at first rapid, then more gradual; the same is true of the production of acid as shown by titration. The reaction is apparently mono-molecular.

Our experiments favor the following conception of the mode of action of "mustard" upon the living cell. The undecomposed "mustard gas" is slightly soluble in water (according to our titrations of completely hydrolyzed solution to the extent of ca. .05 per cent.). This dissolved "mustard" readily penetrates the cell, presumably because of its high lipoid-water partition-coefficient, and collects in relatively high concentration in the organic solvents of the protoplasm (cell-lipoids, fats, etc.). In this situation it serves as a reservoir of toxic material which continually enters solution in the aqueous phases of the protoplasm and is continually being there decomposed. Since by its hydrolytic decomposition it yields acid, the dissolved "mustard" acts destructively on the protoplasm as soon as the available buffer compounds (which normally prevent protoplasmic hyper-acidity) are exhausted. The destructive action is thus due primarily to the HCl freed by hydrolysis. The other decomposition-products are only slightly toxic; this we have shown experimentally by comparing the action of partially or wholly hydrolyzed solutions of the "mustard," from which the acid was removed by neutralization with NaOH, with that of the unneutralized solution. The latter solution is always by far the more toxic;

removal of the acid thus removes the greater part of the toxicity. The continued intracellular production of acid from the reserve of lipoid-bound "mustard" renders the compound, once it has penetrated the cell, extremely persistent in its action and difficult to counteract.

The toxic action of "mustard gas" has a prolonged latency, a fact in accordance with the above conception. Fertilized starfish eggs treated for a few minutes (up to eight minutes) with a freshly prepared weak solution of "mustard" continue to cleave for some hours, at first regularly; later the cleavage becomes irregular and the eggs break down and disintegrate. If acid derived from the progressive hydrolysis of "mustard" contained as reserve in the cell-lipoids is chiefly responsible for the toxic effect, the long latent period of action is readily understood. An experiment with adult fish (*Fundulus*) illustrates both the long latent period and the necessity that the "mustard" should be absorbed by the living cells while it is still in the intact or non-hydrolyzed state. Four fish were placed in each of the following solutions: (A) Filtered solution of "mustard" kept at room temperature five days; (B) a similar solution kept at room temperature one day; (C) the same solution as B, but kept at 0° C. and brought to 20° C. one half hour before using; (D) the same solution kept at 0° C. until immediately before using.

Solutions A and B were almost non-toxic; three of the four fish remained alive after five days in the solution; in C all fish were living after three hours, three were dead in eighteen hours, and all in twenty-six hours; in D two were dead and a third dying within three hours. The toxicity is thus an inverse function of the time during which the "mustard" is undergoing hydrolysis.

While the loss of toxicity of an aqueous "mustard" solution corresponds roughly with the decomposition of "mustard" as determined by titration, a lag in loss of toxicity at the end of the curve suggests that in those extremely dilute solutions the organism takes up a larger proportion of the poison than would be anticipated, possibly as a result of adsorption.

The velocity of the toxic action exhibits a high temperature-coefficient similar to that of chemical reactions in general. In one experiment freshly fertilized starfish eggs were placed in two portions of the same "mustard" solution, one (A) kept at 9 to 10°, the other (B) at 21°. From each solution eggs were transferred to normal sea-water after exposures of 1, 2, 4 and 8 minutes. It was found that an exposure of 2–4 minutes at 21° had almost the same effect in preventing development as one of 8 minutes at 9°–10°. All eggs were killed by 8 minutes' exposure at 21°, while most survived this exposure at 9°. The rate of toxic action at 9°–10° is thus about one third of that at 21°. This result suggests that *cold,* in conjunction with the other methods of treatment, may prove to be of service in treating the skin-burns caused by "mustard gas," *i. e.,* it indicates that the temperature of the skin should be kept as low as possible during the treatment (*e. g.,* washing with ice-cold kerosene is suggested).

Experiments on the counteraction of the toxic action by subsequent treatment with weak basic substances which readily penetrate protoplasm (ammonia, aniline) have not yielded very conclusive results. In several experiments fertilized eggs exposed to "mustard" solutions for some minutes and then brought for three or four hours into sea-water containing a little ammonia $n/2000$ (NH_3 in sea-water) showed on the whole a more favorable development than eggs returned directly from the "mustard" solution to sea-water (*i. e.,* larvæ showed less irregularity and more active ciliary movement). This favorable effect of ammonia was distinct but somewhat slight. In other experiments *Arenicola* larvæ treated for some minutes with solutions of aniline in sea-water (of the anæsthetizing concentration, ca. 1/8 saturated), and then exposed to "mustard" solution, proved distinctly more resistant to its toxic action than the control. This effect is probably to be regarded as an example of the general protective or antitoxic action which anæsthetics exhibit

with this organism. It is possible, however, that the basicity of aniline may be favorable; larvæ anæsthetized with alcohols showed some degree of protection, but less marked than with aniline. The after-treatment of poisoned larvæ with aniline solutions proved ineffective.

Treatment with basic substances appears to us to offer the most promising means of counteracting the action of this poison. A substance whose physical properties, solubilities, and rate of hydrolysis resemble those of "mustard," but which yields on hydrolysis a *base, e. g.,* ammonia, instead of an acid, ought theoretically to counteract the action of "mustard" within the cell. Such a compound could be introduced into the lungs in the form of a spray, or applied to the skin in the usual manner. High lipoid-solubility or surface-activity, favoring rapid penetration of cells, would be essential in such a substance. We recommend a systematic search for an organic compound having these properties. Physiological experimentation with such a compound, if it is obtainable, should in our opinion yield important results.

By the use of intravitam staining, and by the injection of aqueous "mustard" solution directly into the body of the starfish egg, strong evidence was afforded that free acid is liberated within the cell.

The intravitam stain used was neutral red. Eggs were treated with solutions of "mustard" oil (in sea-water) sufficiently concentrated to cause subsequent abnormal development, and were then transferred to an extremely dilute solution of neutral red in sea-water. Normal eggs were simultaneously treated with the neutral red solution. For a period of at least half an hour controlled and treated eggs were colored to about the same degree. The treated eggs later became progressively more intensely stained, so that in an hour after the treatment the greater intensity in color of the "gassed" eggs over that of the control was easily recognizable.

The effect of "mustard" and its decomposition-products on the cell-interior was tested by the introduction of a drop of the gas solution into the body of the fertilized egg by means of a micro-pipette. The following results were obtained:

1. Eggs injected with distilled water quickly recover and continue their normal development.

2. Eggs injected with a freshly made saturated aqueous solution of "mustard gas" show no immediate injurious effects but subsequently are inhibited in their development.

3. Eggs injected with a saturated solution which has been allowed to stand at room temperature for over two hours undergo cytolysis, the immediate destructive effect being more marked than that following the injection of the undecomposed solution.

4. Eggs injected with an aqueous solution of hydrochloric acid of the same strength as the decomposed gas solution exhibit approximately the same effect, viz., a more or less extended cytolysis.

These experiments lend substantial support to the view, previously expressed by Marshall and Smith, that mustard gas, in virtue of its lipoid-solubility, penetrates rapidly into the cell-interior where it liberates hydrochloric acid which, in the free state, is relatively incapable of penetrating the cell.

R. S. LILLIE,
G. H. A. CLOWES,
R. CHAMBERS
THE MARINE BIOLOGICAL LABORATORY,
WOOD'S HOLE, MASS.

SPECIAL ARTICLES

ON HERSCHELL'S FRINGES

HERSCHELL'S fringes, as produced by the familiar apparatus consisting of a right-angled prism reposing with its broad face on a plate of obsidian, present the well-known group of achromatic fringes running parallel to the arc or limit of total reflection. Observation is made in a direction normal to the edge of the prism.

It occurred to me that the phenomenon could be made much more striking and of wider scope, if a long 60° prism were used and observation made in a plane of symmetry *parallel* to the edge of the prism. In the interest of variety, moreover, it is preferable not to em-